Protein-Protein and Protein-DNA interactions by switchSENSE: comparison with other approaches

Paloma FERNANDEZ VARELA

I2BC : B3S department, MiKiCa team - PIM platform

Warsaw 2018 ARBRE-MOBIEU Plenary Meeting

Flash presentation: Protein Structure, Interactions & Functions

switchSENSE®

How does switchSENSE fit into the toolbox of classical biophysical methods?

The « classics »	gel electrophoresis (EMSA), ELISA	binding y/n, concentration dep.
Solution methods	ITC, FP, fluorescence, thermal shift, stopped flow, affinity chromatography (KinExA), MST, DSC,…	k_D from equilibrium T_M, k_{ON}
Surface biosensors	SPR, BLI, waveguides,… (refractive index sensors)	k_{ON}, k_{OFF}, k_D from kinetics k_D from equilibrium
Brownian motion	DLS, FCS,…	diffusion coeff. & Stokes diameter
Structure	NMR, XRD, SAXS,…	atomic structure, binding

switchSENSE

switchSENSE®

Accessible biophysical parameters

BINDING

k_{ON}

K_D

k_{OFF}

avidity

SIZE, SHAPE

Stokes diameter D_H

dimers, trimers, aggreg.

conf. changes

K_M

k_{CAT}

ENZYMATIC ACTIVITY

THERMO-DYNAMICS

stability

T_M

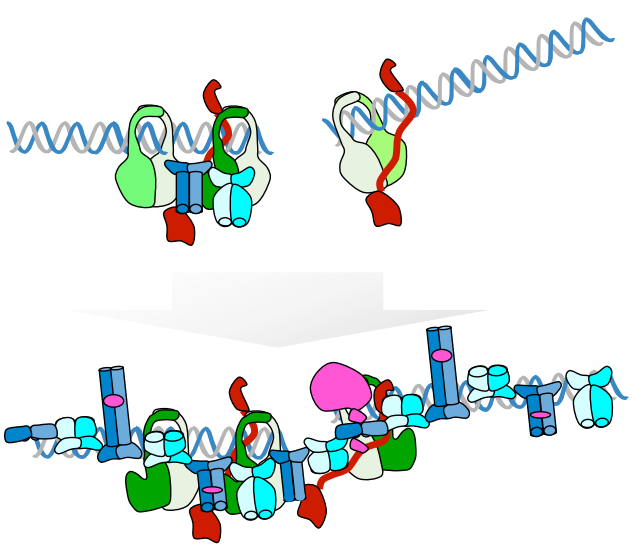
ΔH

ΔG

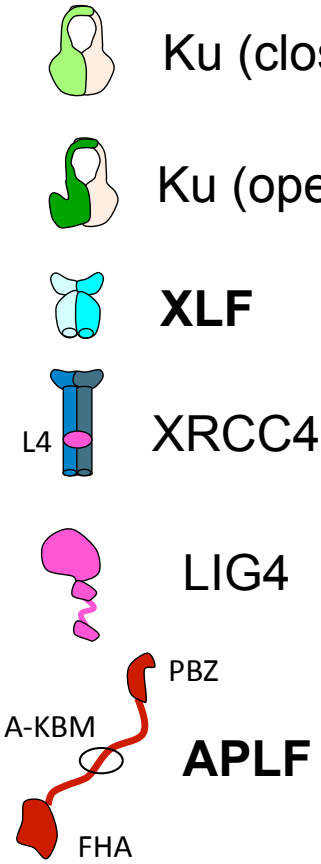
ΔS

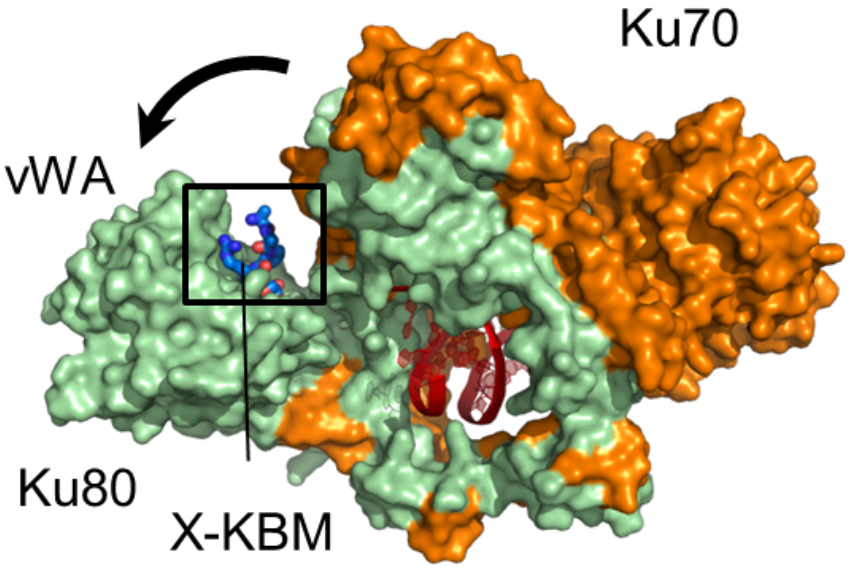
Project 1: Ku

- **Context :** Human Double-Strand Break Repair Pathway
- **Interaction:** Ku70/Ku80 (recognition step) and XLF (ligation step)



SPR, ITC, MST

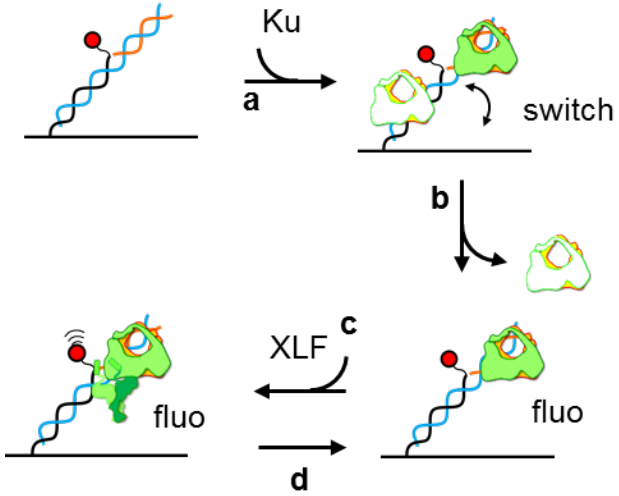


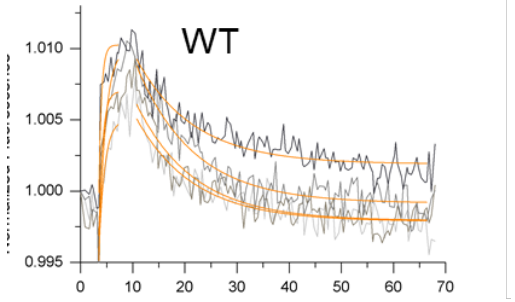


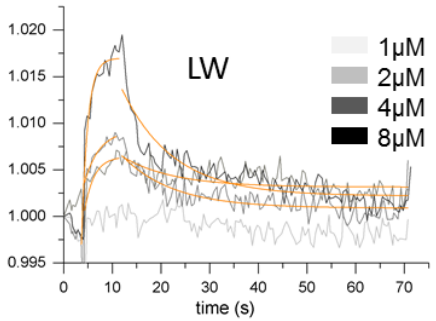
JB Charbonnier (I2BC, CEA Saclay)

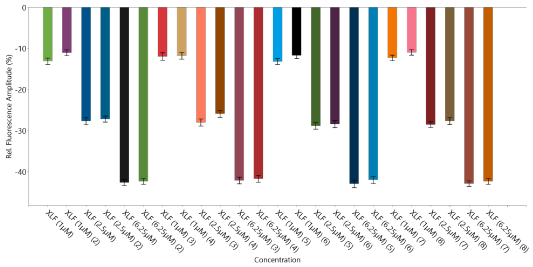
Project 1: Ku (2)

- **Conclusions:** Kinetics of Ku-DNA and Ku-XLF and mutants









- **Rapid association and dissociation**
- **Change in fluorescence amplitude upon binding**

XLF(WT) $k_D = 0.19 \pm 0.07$ µM $k_{ON} = 4.7 \pm 1.7\ 10^5\ M^{-1}s^{-1}$ and $k_{OFF} = 9.1 \pm 0.4\ 10^{-2}\ s^{-1}$
XLF(LW) $k_D = 0.45 \pm 0.26$ µM $k_{ON} = 1.9 \pm 1.1\ 10^5\ M^{-1}s^{-1}$ and $k_{OFF} = 8.4 \pm 0.6\ 10^{-2}\ s^{-1}$
XLF(LE) $k_D = 0.98 \pm 0.15$ µM $k_{ON} = 4.9 \pm 0.5\ 10^4\ M^{-1}s^{-1}$ and $k_{OFF} = 4.8 \pm 0.5\ 10^{-2}\ s^{-1}$

PLENTY OF POSIBILITIES FOR DNA BINDING PROTEINS

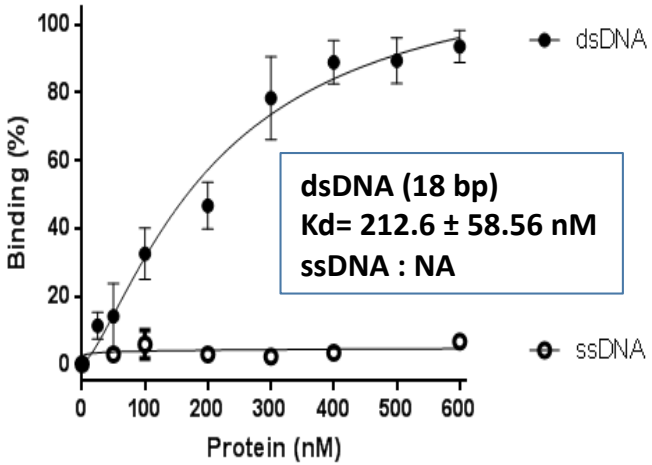
Project 2: ComH

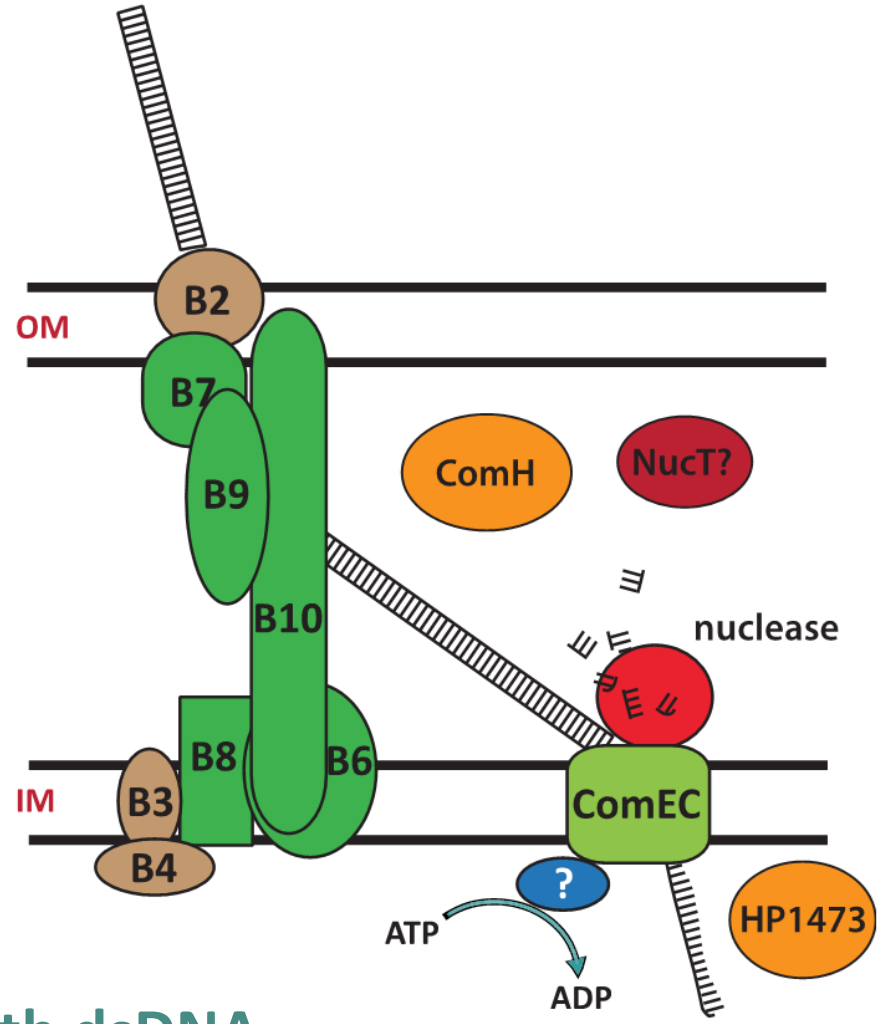
- **Context :**

Transformation in *H Pylori*

- **Interaction:**

ComH (new factor) and dsDNA or ssDNA (transformation products)





EMSA

Interaction with dsDNA, but not with ssDNA

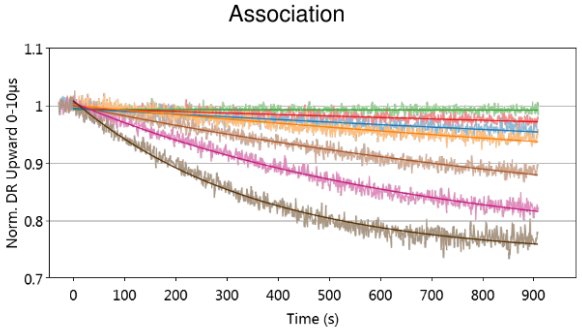
P Radicella, P Damke (CEA, Fontenay aux Roses)

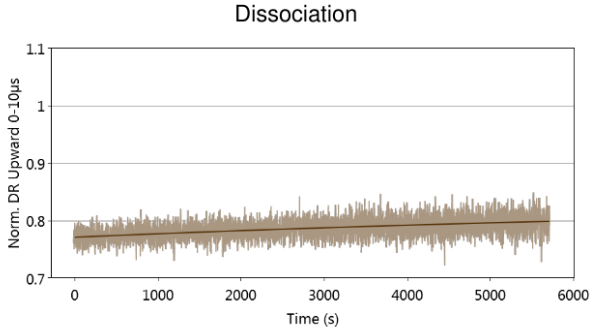
Project 2: ComH (2)

switchSENSE®

dsDNA

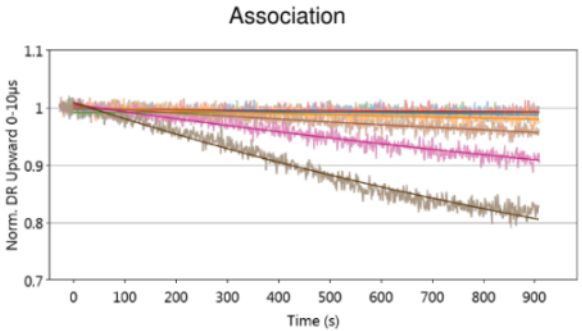
green ds DNA on the chip (NLA48+cNLA48)
Protein concentration:
0,6nM to 20nM

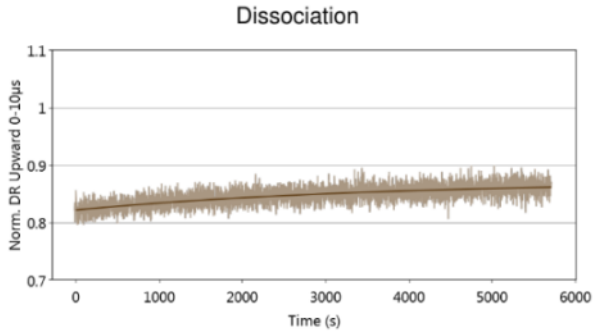




ssDNA

red ss DNA on the chip DNA (NLB48)
Protein concentration:
0,6nM to 20nM



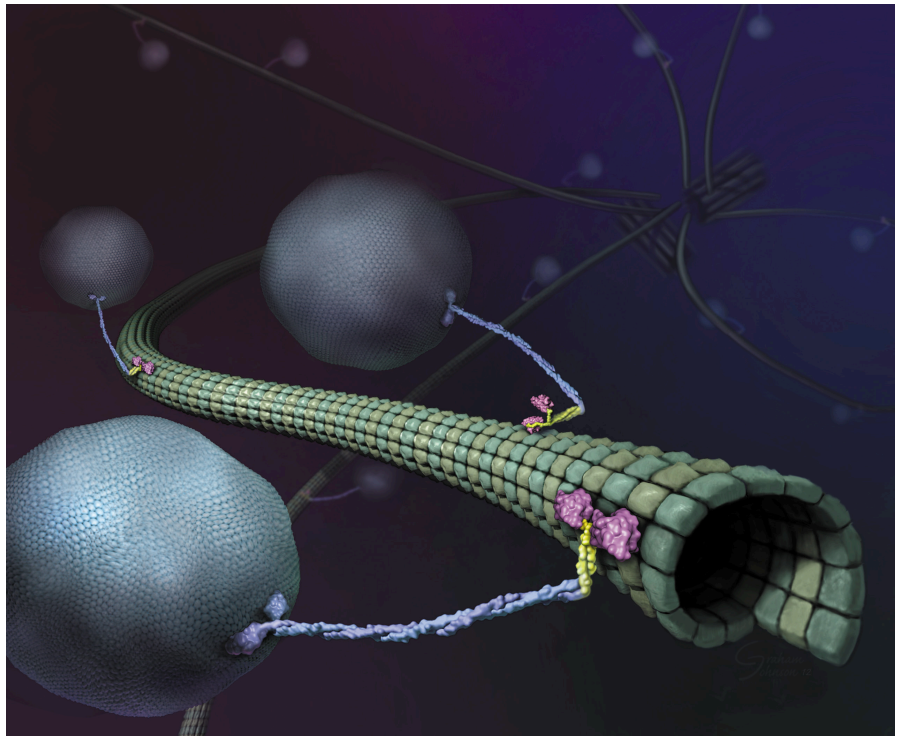


	48ds			48ss		
	MBP-ComH	MBP-ComH-NTD	MBP	MBP-ComH	MBP-ComH-NTD	MBP
kd (nM)	**0,70** +/- 0,25	**2,11** +/- 0,14	-	**15,2** +/- 2,0	**0,929**+/-0,039	-
k_{on} ($M^{-1}.s^{-1}$)	**1,36** +/- 0,02.**10^{+5}**	**2,48** +/- 0,13.**10^{+5}**	-	**1,75** +/- 0,19.**10^{+4}**	**3,63**+/-0,06.**10^{+5}**	-
k_{off} (s^{-1})	**9,58** +/- 3,42.**10^{-5}**	**5,23** +/- 0,20.**10^{-4}**	-	**2,79** +/- 0,20.**10^{-4}**	**3,37**+/-0,13.**10^{-4}**	-

TWO EXPERIENCES IN A SINGLE RUN

Project 3 : GAPDH

- **Context :** Molecular motors in Fast Axonal Transport
- **Interaction:** GAPDH (ATP production) and KLC^{TPR6} domain of Kinesin-1

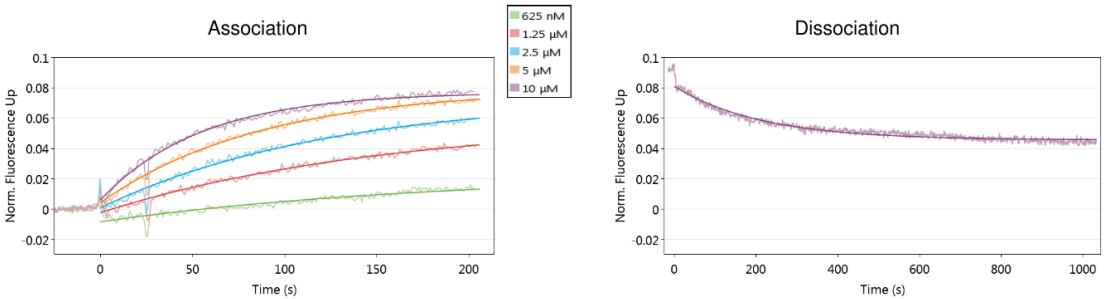


AUC, ITC, SEC-MALS
MST, SPR

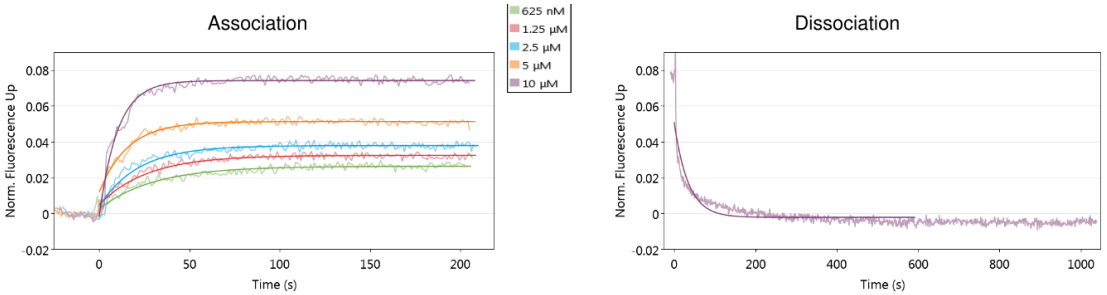
J Ménétrey, PF Varela (I2BC, CNRS Gif sur Yvette)

Project 3 : GAPDH (2)

HisKLC^{TPR6}/HisGAPDH



HisGAPDH/HisKLC^{TPR6}



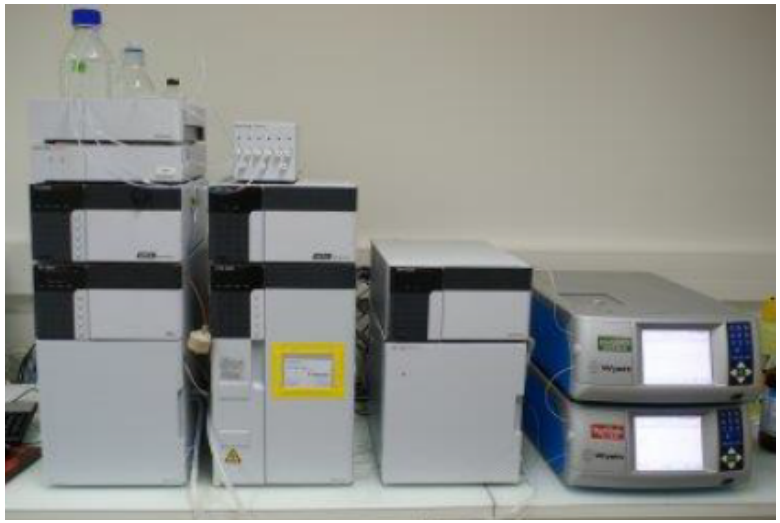
	Kinetics triplicate low & high concentration (µM)	Kinetics titration + Zero subtraction (µM)
His-KLC / GAPDH	1.39 ± 0.04	3.48 ± 0.09
His-GAPDH/ KLC	0.25 ± 0.01	4.21 ± 0.19

CUSTOMIZABLE TO TARGET TYPE, SCIENTIFIC QUESTION

PIM : https://gipsi.i2bc.paris-saclay.fr/

MICROCALORIMETRY

SECMALS



SPR

MST

AUC-FDS

POSTER 12


gado
Dank U
Merci
mahalo
Kö
cuбo
Grazie
Thank you
mauruuru
Tak
Gracias
Dziękuję
Děkuju
danke
Kii